

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTI-¹⁵FOU₁₋₄ BY ANTBODY - Q9E24O2
LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

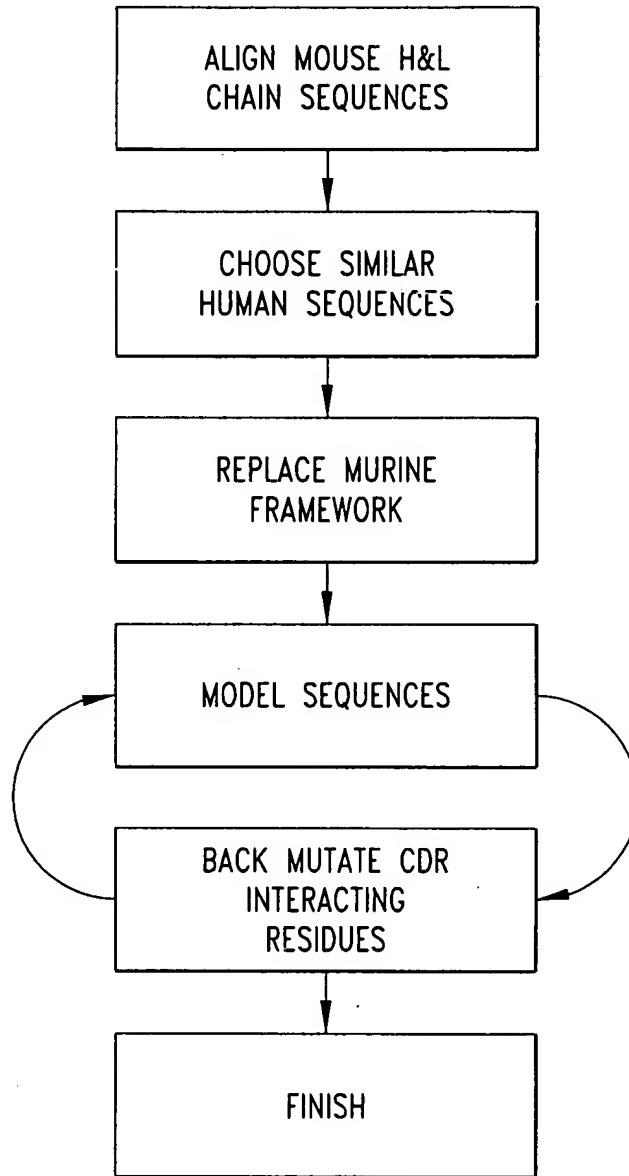


Fig. 1



NR-LU-13 Heavy chain variable region sequences

GAG GTT CAG CTG CAG CAG TCT GGG GCA GAG CTT GTG AAG CCA GGG GCC TCA GTC AGG TTG TCC TGC Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Arg Leu Ser Cys	22
CDR1	
ACA GCT TCT GGC TTC AAC ATT AAA GAC ACC TAT ATG CAC TGG GTG ATA GAG AGG CCT GAA CAG GGC Thr Ala Ser Gly Phe Asn Ile Lys Asp Thr Tyr Met His Trp Val Ile Glu Arg Pro Glu Gln Gly	44
CDR2	
CTG GAG TGG ATT GGA AGG ATT GAT CCT GCG AAT GGT AAT ACT AAA TGT GAC CCG AAG TTC CAG GGC Leu Glu Trp Ile Gly Arg Ile Asp Pro Ala Asn Gly Asn Thr Lys Cys Asp Pro Lys Phe Gln Gly	66
AAG GCC ACT ATA ACA GCA GAC ACA TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT Lys Ala Thr Ile Thr Ala Asp Thr Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser	88
CDR3	
GAG GAC ACT GCC GTC TAT TAC TGT TCT AGA GAG GTC CTA ACT GGG ACG TGG TCT TTG GAC TAC TGG Glu Asp Thr Ala Val Tyr Tyr Cys Ser Arg Glu Val Leu Thr Gly Thr Trp Ser Leu Asp Tyr Trp	110
GGT CAA GGA ACC TCA GTC ACC GTC TCC TCA Gly Gln Gly Thr Ser Val Thr Val Ser Ser	120

NR-LU-13 Light chain variable region sequences

GAC ATC CAG ATG ATT CAG TCT CCA TCG TCC ATG TTT GCC TCT CTG GGA GAC AGA GTC AGT CTC TCT Asp Ile Gln Met Ile Gln Ser Pro Ser Ser Met Phe Ala Ser Leu Gly Asp Arg Val Ser Leu Ser	22
CDR1	
TGT CGG GCT AGT CAG GGC ATT AGA GGT AAT TTA GAC TGG TAT CAG CAG AAA CCA GGT GGA ACT ATT Cys Arg Ala Ser Gln Gly Ile Arg Gly Asn Leu Asp Trp Tyr Gln Gln Lys Pro Gly Gly Thr Ile	44
CDR2	
AAA CTC CTG ATC TAC TCC ACA TCC AAT TTA AAT TCT GGT GTC CCA TCA AGG TTC AGT GGC AGT GGG Lys Leu Leu Ile Tyr Ser Thr Ser Asn Leu Asn Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly	66
TCT GGG TCA GAT TAT TCT CTC ACC ATC AGC AGC CTA GAC TCT GAA GAT TTT GCA GAC TAT TAC TGT Ser Gly Ser Asp Tyr Ser Leu Thr Ile Ser Ser Leu Asp Ser Glu Asp Phe Ala Asp Tyr Tyr Cys	88
CDR3	
CTA CAG CGT AAT GCG TAT CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA Leu Gln Arg Asn Ala Tyr Pro Tyr Phe Gly Gly Thr Lys Leu Glu Ile Lys	107

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY NRX451
NRX-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

Light Chain



1	5	10							
ASP	ILE	GLN	MET	THR	GLN	SER	PRO	SER	SER
11	15	20							
LEU	SER	ALA	SER	VAL	GLY	ASP	ARG	VAL	THR
21	25	30							
ILE	THR	CYS	ARG	ALA	SER	GLN	GLY	ILE	ARG
31	35	40							
GLY	ASN	LEU	ASP	TRP	TYR	GLN	GLN	LYS	PRO
41	45	50							
GLY	LYS	GLY	PRO	LYS	LEU	LEU	ILE	TYR	SER
51	55	60							
THR	SER	ASN	LEU	ASN	SER	GLY	VAL	PRO	SER
61	65	70							
ARG	PHE	SER	GLY	SER	GLY	SER	GLY	SER	ASP
71	75	80							
TYR	THR	LEU	THR	ILE	SER	SER	LEU	GLN	PRO
81	85	90							
GLU	ASP	PHE	ALA	THR	TYR	TYR	CYS	LEU	GLN
91	95	100							
ARG	ASN	ALA	TYR	PRO	TYR	THR	PHE	GLY	GLN
101	105								
GLY	THR	LYS	LEU	GLU	ILE	LYS			

The humanized sequence of NRX451 light chain,
residue positions which differ between NR-LU-13
and NRX451-humanized are marked with bold
type.

Fig. 3

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY AN antibody 1092402

NR-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

Heavy Chain



1	5	10							
GLN	VAL	GLN	LEU	VAL	GLN	SER	GLY	ALA	GLU
11	15	20							
VAL	LYS	LYS	PRO	GLY	ALA	SER	VAL	LYS	VAL
21	25	30							
SER	CYS	LYS	ALA	SER	GLY	PHE	ASN	ILE	LYS
31	35	40							
ASP	THR	TYR	MET	HIS	TRP	VAL	ARG	GLN	ALA
41	45	50							
PRO	GLY	GLN	GLY	LEU	GLN	TRP	MET	GLY	ARG
51	55	60							
ILE	ASP	PRO	ALA	ASN	GLY	ASN	THR	LYS	CYS
61	65	70							
ASP	LEU	SER	PHE	GLN	GLY	ARG	VAL	THR	ILE
71	75	80							
THR	ALA	ASP	THR	SER	ILE	ASN	THR	ALA	TYR
81	85	90							
MET	GLU	LEU	SER	SER	LEU	ARG	SER	ASP	ASP
91	95	100							
THR	ALA	VAL	TYR	TYR	CYS	SER	ARG	GLU	VAL
101	105	110							
LEU	THR	GLY	THR	TRP	SER	LEU	ASP	TYR	TRP
111	115	120							
GLY	GLN	GLY	THR	LEU	VAL	THR	VAL	SER	SER

The humanized sequence of NRX451 light chain, residue positions which differ between NR-LU-13 and NRX451-humanized are marked with bold type.

Fig. 4

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 1D9E2402
NR-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2



Alignment of the Light Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

DIQMISSPSSMFASLGDRVSLSC RASQGIRGNLD WYQQKPGGTIKLLIY STSNLNS

.....
DIQMTQPSSLSASVGDRVITTC RASQGIRGNLD WYQQKPGKGPKLLIY STSNLNS
CDR1 CDR2

GVPSRFSGSGSGSDYSLTISSLESEDFA~~D~~YYC LQRNAYPYTF GGGTKLEIK

.....
GVPSRFSGSGSGSDYTLTISSLQPEDFAT~~A~~YYC LQRNAYPYTF GQGTKLEIK
CDR3

Alignment of the Heavy Chain Variable Regions of
NR-LU-13 (top) and humanized NRX451 (bottom).

EVQLQSGAELVKPGASVRLSCTASGFNIK DTYMH WVIERPEQGLEWIG

.....
QVQLVQSGAEVKPGASVKVSCKASGFNIK DTYMH WVRQAPGQGLQWMG
CDR1

RIDPANGNTK CDPKFQGKATITADTSSNTAYLQLSSLTSEDTVYYCS

.....
RIDPANGNTK CDLSFQGRVTITADTSINTAYMELSSLRSDDTAVYYCS
CDR2

REVLTGTVSLDY WGQGTSVTVSS

.....
REVLTGTVSLDY WGQGTLVTVSS
CDR3

Fig. 5

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 1056,794-092402
LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

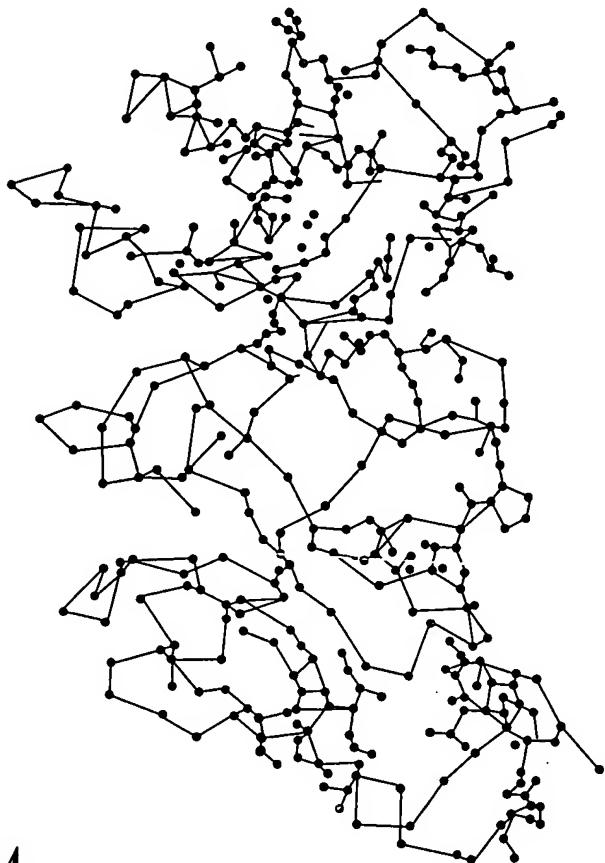


Fig. 6A

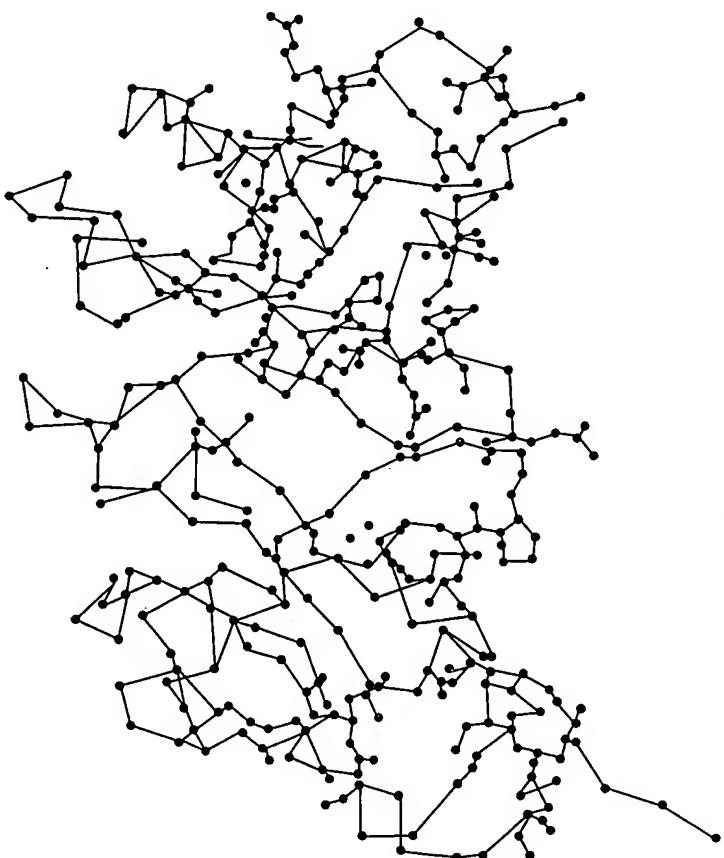


Fig. 6B

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BO~~UN~~^{UN}ND BY ANTIBODY . 092402
LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

Same frequencies, but matching with human sequences. Only one occurrence of Asp at position 182 is found and no occurrences of Cys at position 181.



RES	181	182
A	-	0.48
R	-	0.02
N	0.01	0.18
D	0.00	0.00
C	0.00	0.00
Q	0.00	-
E	-	-
G	0.00	0.01
H	0.00	-
I	-	0.00
L	-	0.00
K	0.00	0.00
M	-	-
F	0.03	-
P	0.00	0.01
S	0.01	0.23
T	-	0.02
W	0.00	-
Y	0.91	-
V	0.00	0.02
X	0.01	0.02
O	-	-
-	-	-
Z	-	-
B	-	0.00
Total	1.00	1.00

Fig. 7A

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY AN antibody . 092402
-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

Sequence positions 50 and 183 are structural mutations within 5 Å of the CDR's. Frequency of residue types at these positions are identical.



Position 50 (153 human lambda sequences)

RES 50

A	-
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	-
K	-
M	0.00
F	-
P	0.93
S	-
T	-
W	-
Y	-
V	-
X	0.06
O	-
-	-
Z	-
B	-

Total 1.00

Fig. 7B

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 100256784092402
-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2



Position 50 (279 human kappa sequences)

RES	50
A	0.00
R	-
N	-
D	-
C	-
Q	-
E	-
G	-
H	-
I	0.00
L	0.00
K	-
M	-
F	-
P	0.96
S	-
T	-
W	-
Y	-
V	-
X	0.03
O	-
-	-
Z	-
B	-
Total	1.00

Fig. 7C

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

Position 50 is highly conserved in all the sequences, but proline can be exchanged by Ile or Leu. The framework used for the light chain (6fab) does have an Ile at this position. If this position is compared to other structures the backbone torsions are the same for structures with a Pro and an Ile at this position.

Position 50 (153 human lambda sequences)

RES	183
A	0.06
R	-
N	0.00
D	0.21
C	-
Q	0.15
E	0.01
G	0.01
H	-
I	0.00
L	0.00
K	0.00
M	-
F	0.00
P	0.40
S	0.01
T	0.01
W	-
Y	0.00
V	0.08
X	0.02
O	-
-	-
Z	-
B	0.00
Total	1.00

Fig. 7D

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY "JG0567794", CIS 24-02
-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2



Position 183 (1210 mouse sequences)

RES 183

A	0.16
R	0.00
N	0.00
D	0.13
C	-
Q	0.16
E	0.25
G	0.02
H	0.00
I	-
L	-
K	0.00
M	-
F	-
P	0.17
S	0.08
T	0.00
W	-
Y	-
V	0.00
X	0.02
O	-
-	-
Z	-
B	-

Total 1.00

Leu is seen in human sequences at this position, but never in murine sequences, for both human and murine Sequences P is the most frequently occurring residue at position 183.

Fig. 7E



Comments for pcDNA3:

5446 nucleotides

CMV promotor: bases 209-863

T7 promotor: bases 864-882

Polylinker: bases 889-994

Sp6 promotor: bases 999-1016

BGH poly A: bases 1018-1249

SV40 promotor: bases 1790-2115

SV40 origin of replication: bases 1984-2069

Neomycin ORF: bases 2151-2945

SV40 poly A: bases 3000-3372

ColE1 origin: bases 3632-4305

Ampicillin ORF: bases 4450-5310

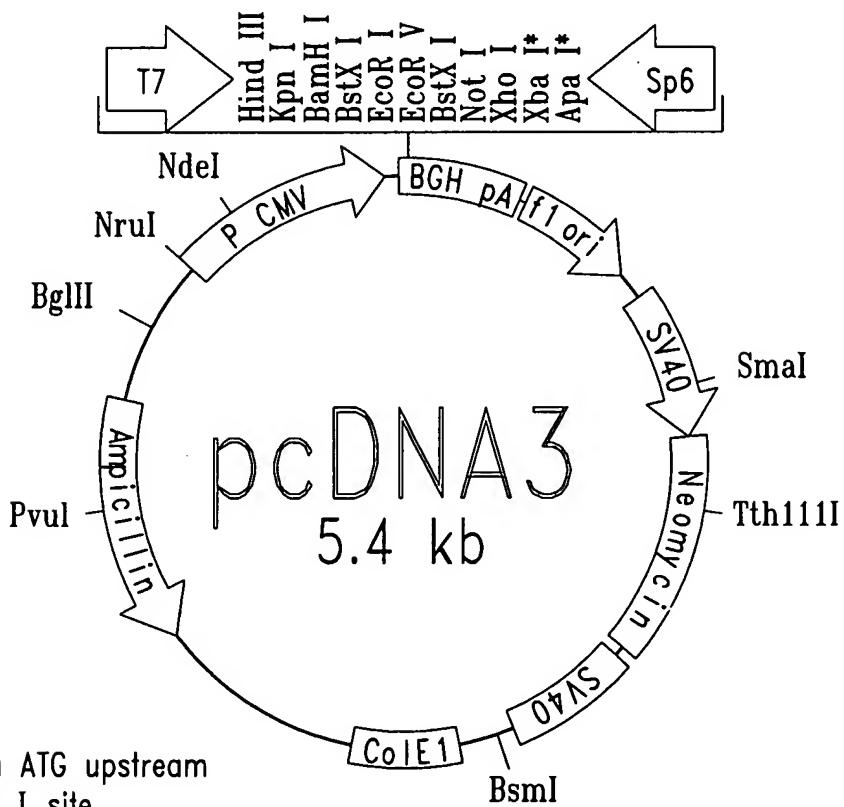


Fig. 8

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY
10/056,794 092402
-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

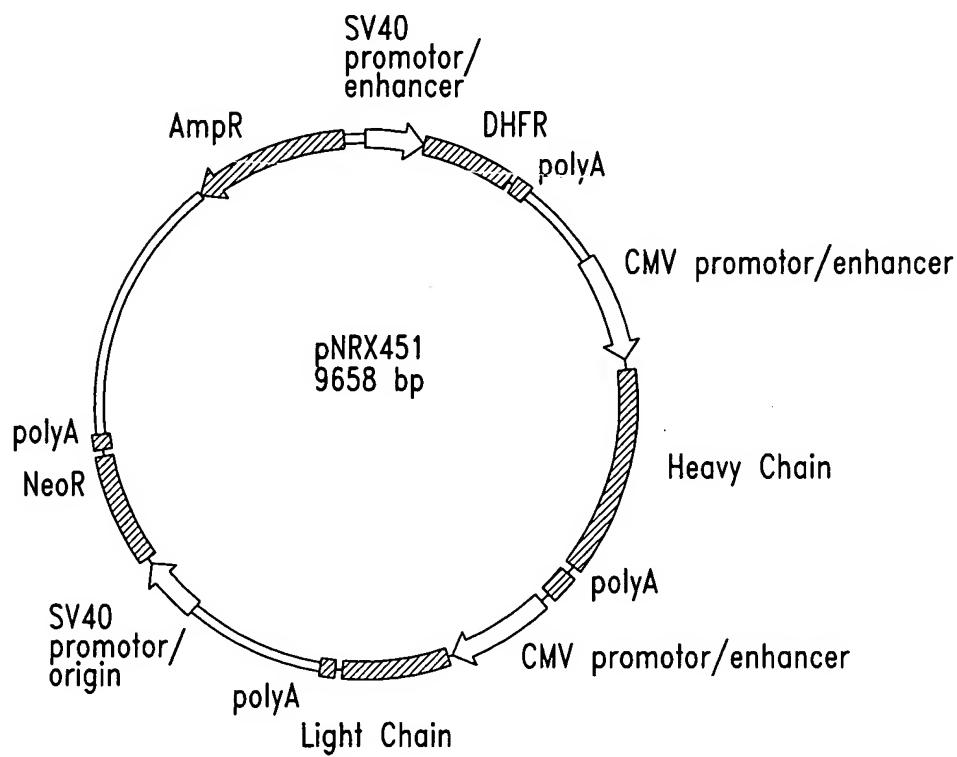


Fig. 9

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY
10056794 092402
-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

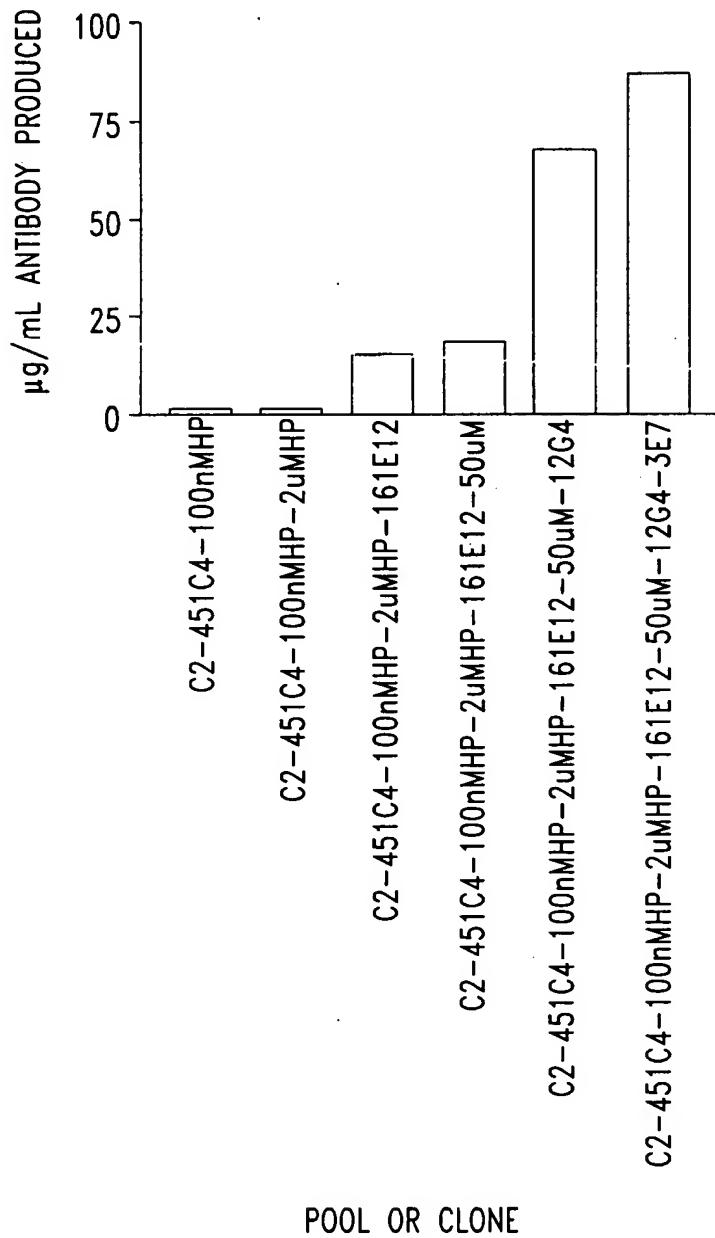


Fig. 10



COMPETITIVE IMMUNOREACTIVITY

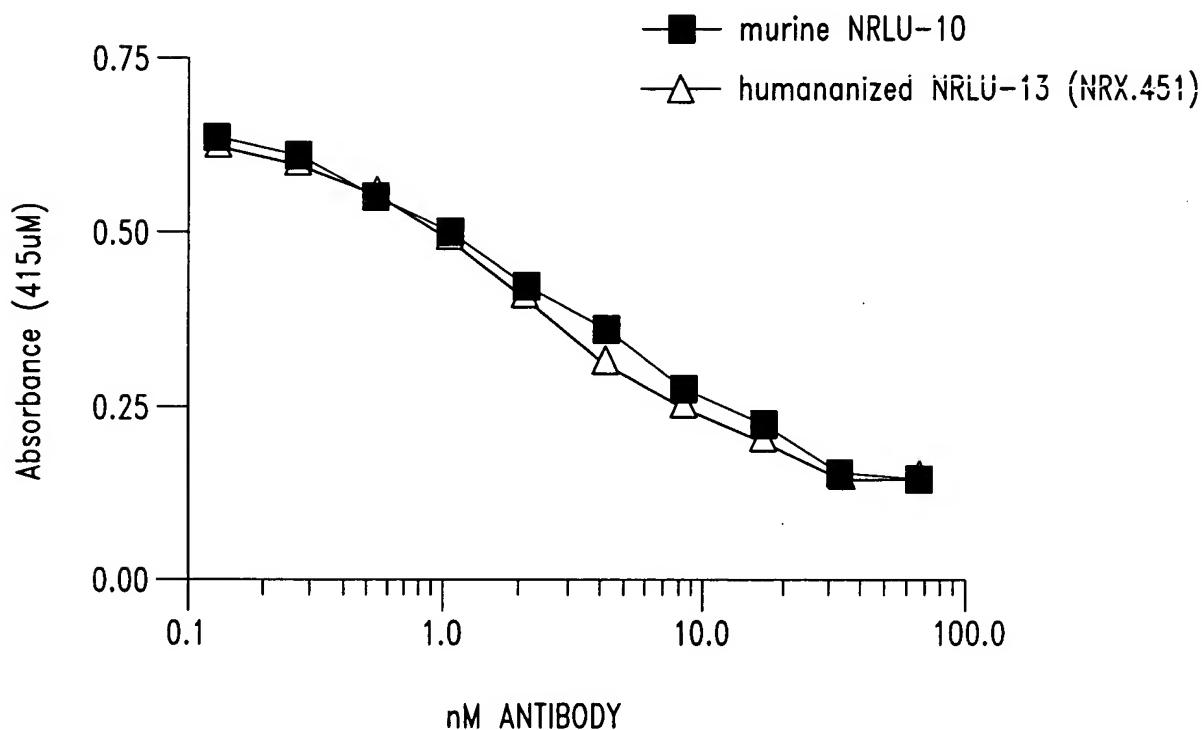


Fig. 11

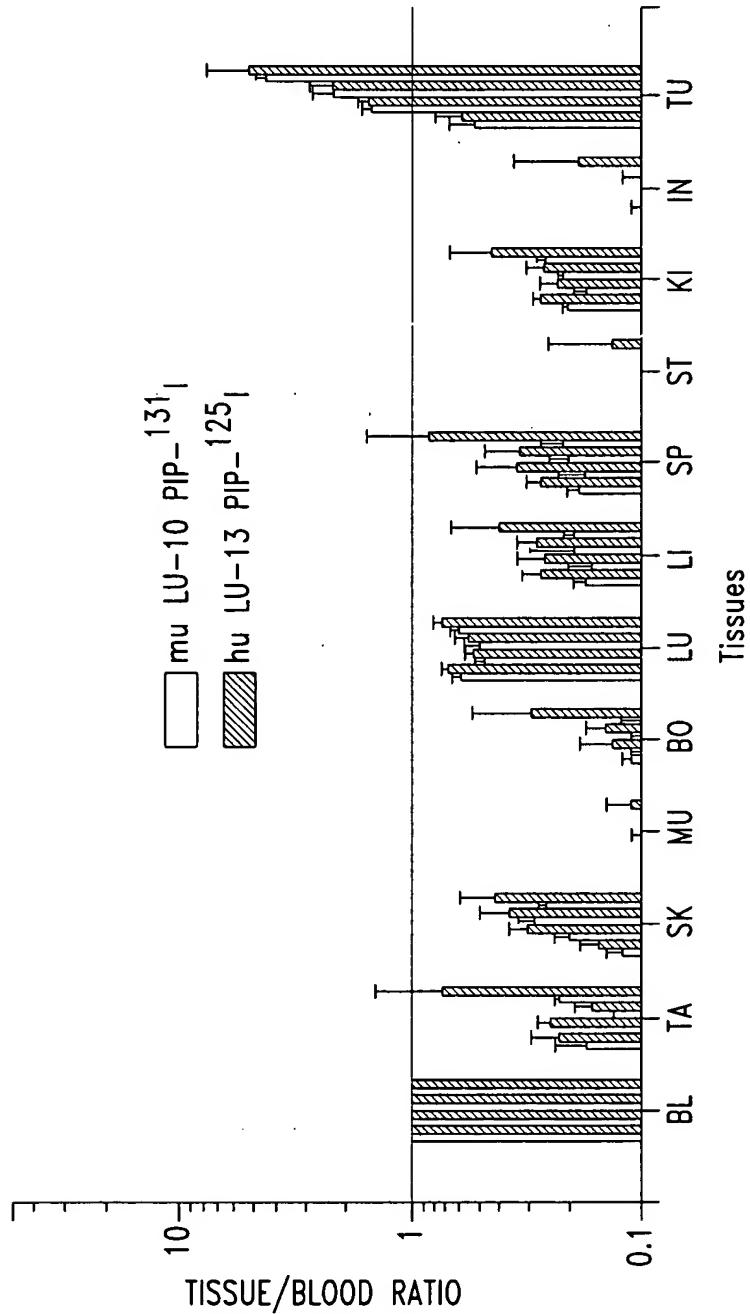


Fig. 12

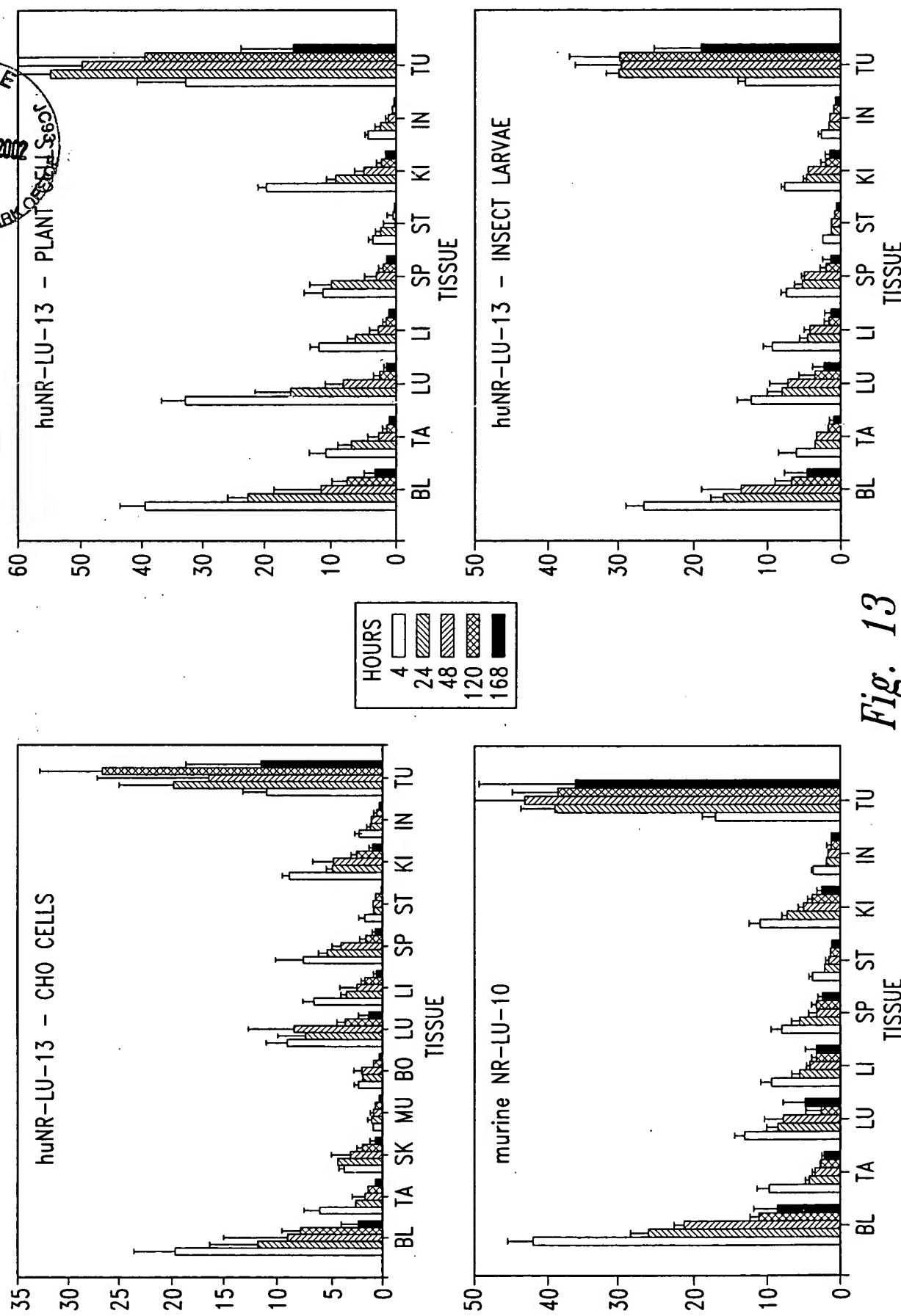


Fig. 13

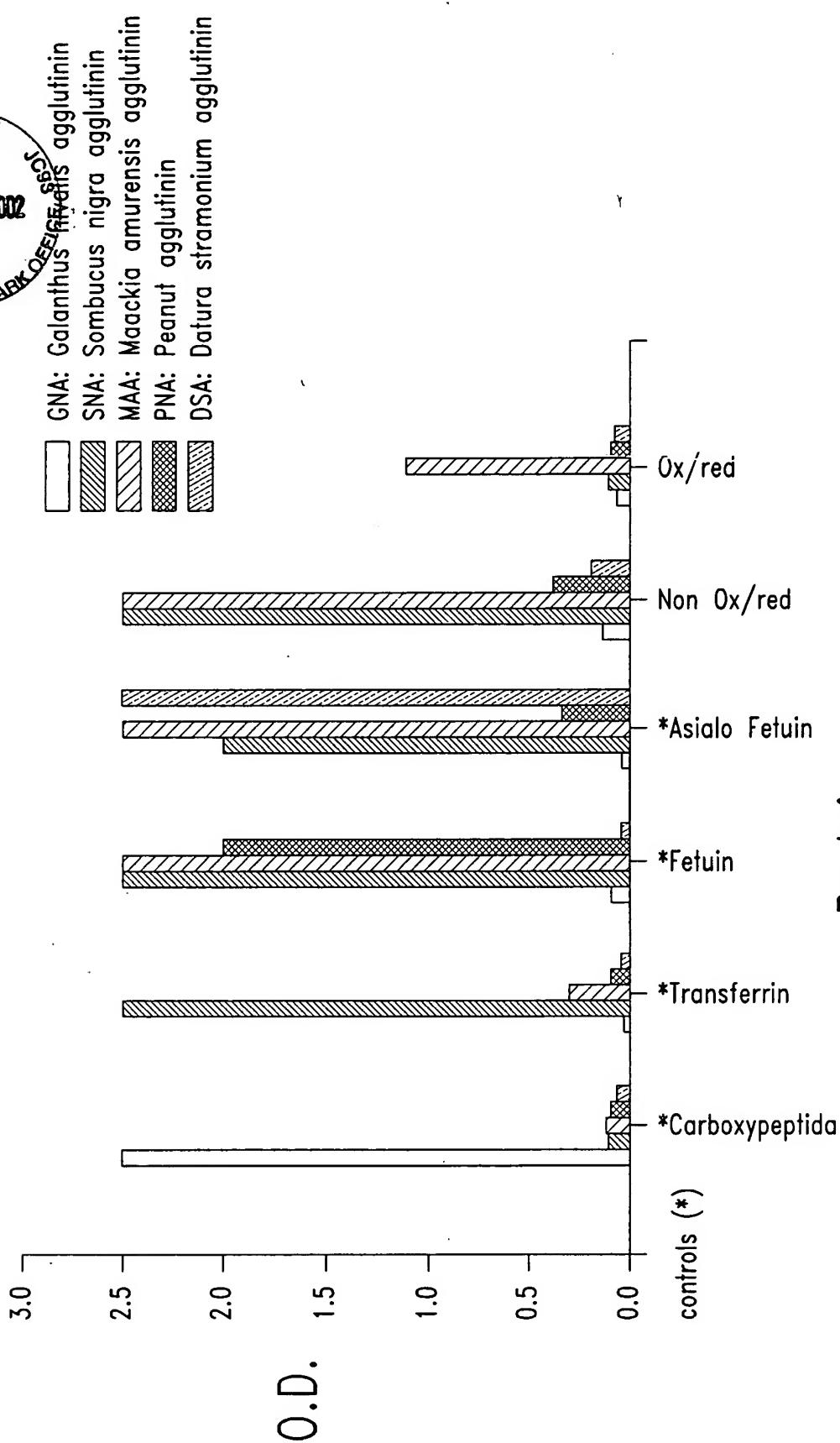


Fig. 14

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY
10056794 · 092402
P-LU-13 AND THEIR USE IN PRETARGETING METHOD
Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

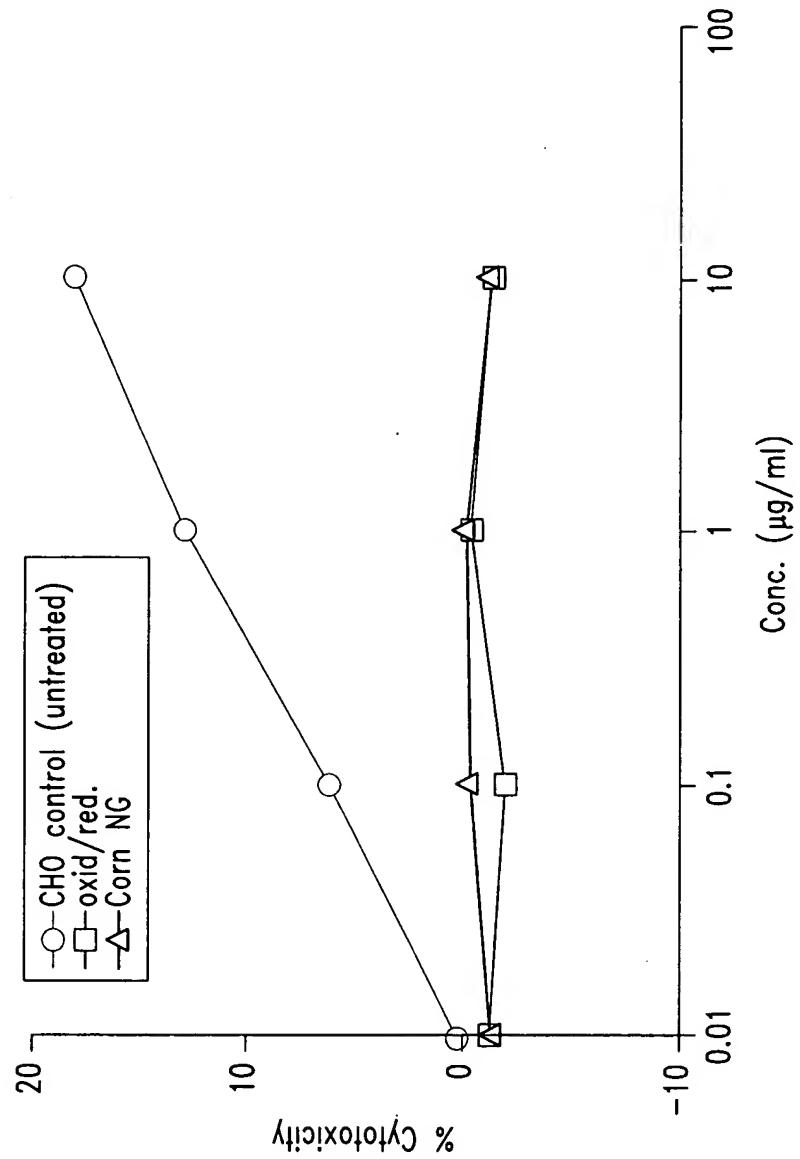


Fig. 15A

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY
-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

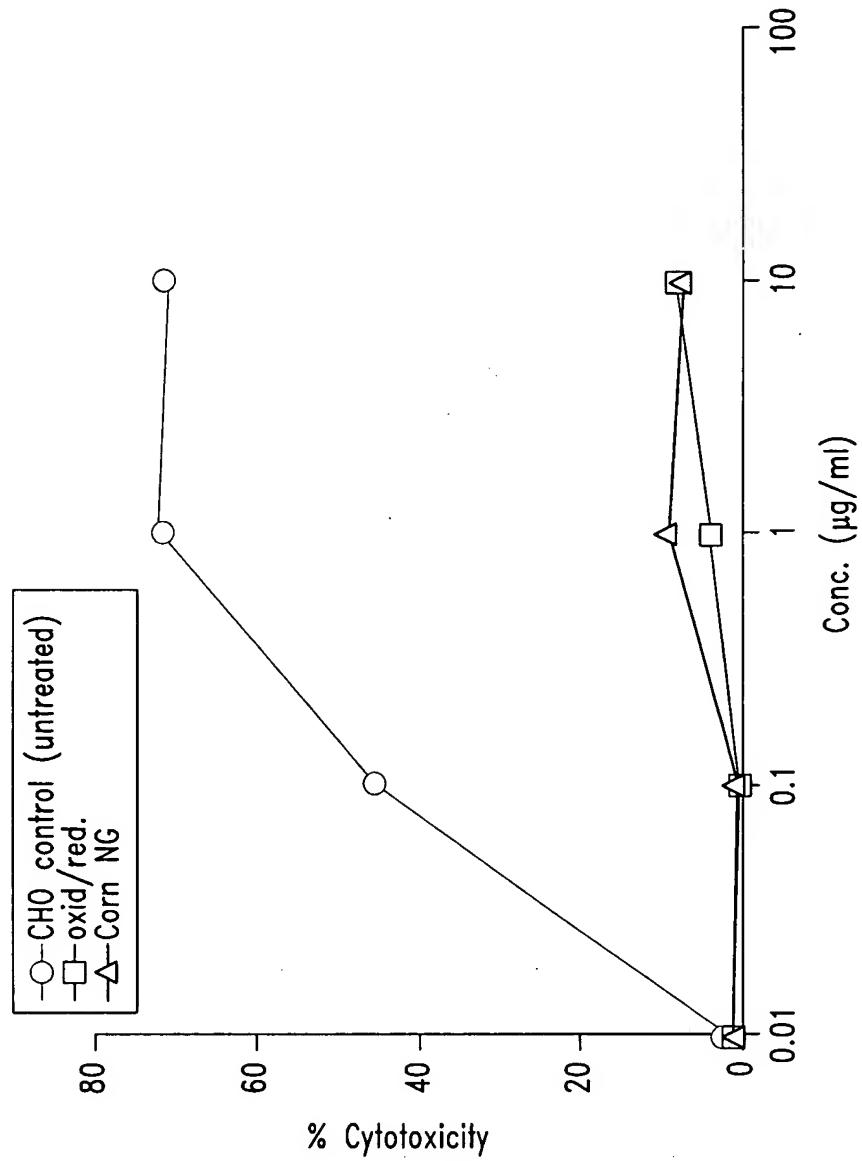


Fig. 15B

10056794 - 092402
Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY
-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

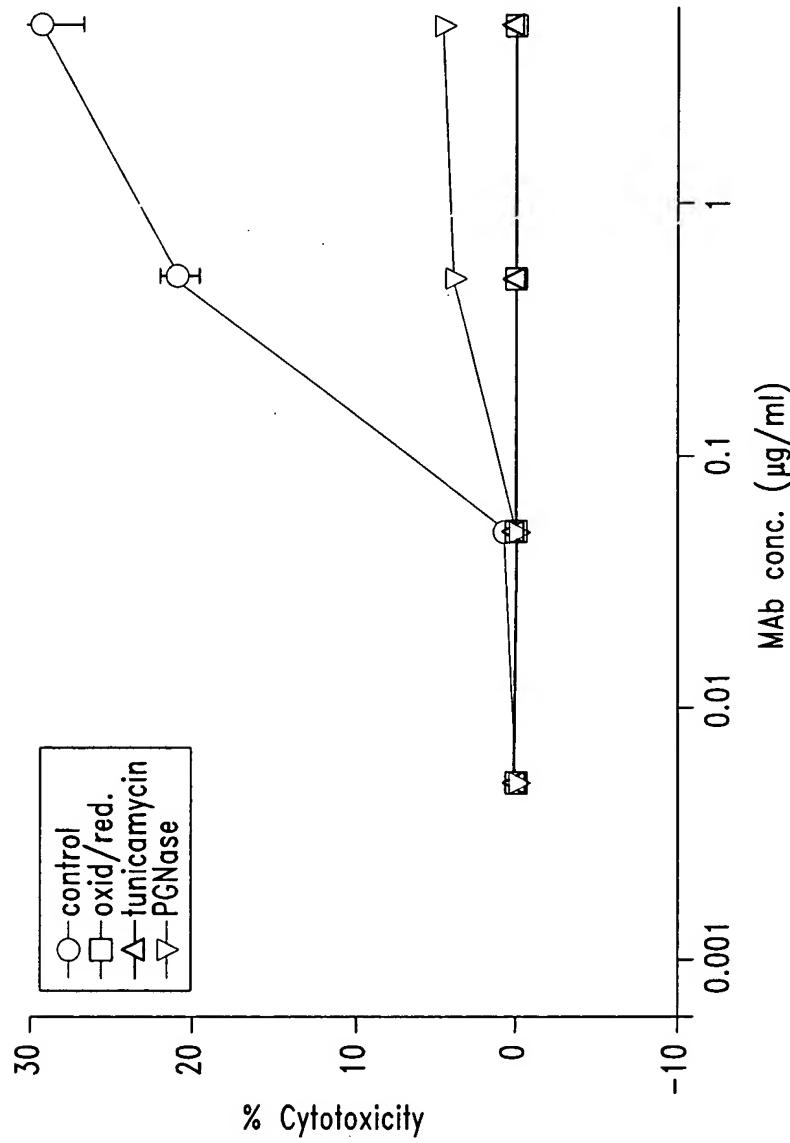


Fig. 15C

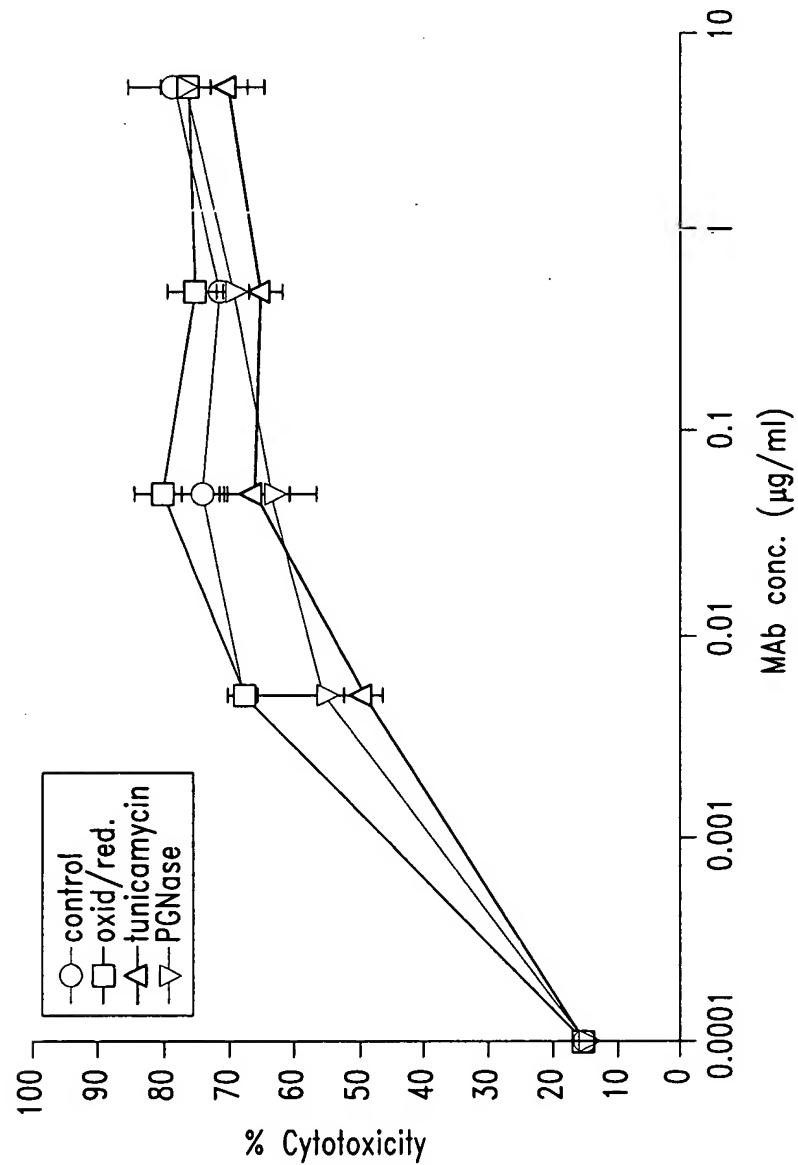


Fig. 15D

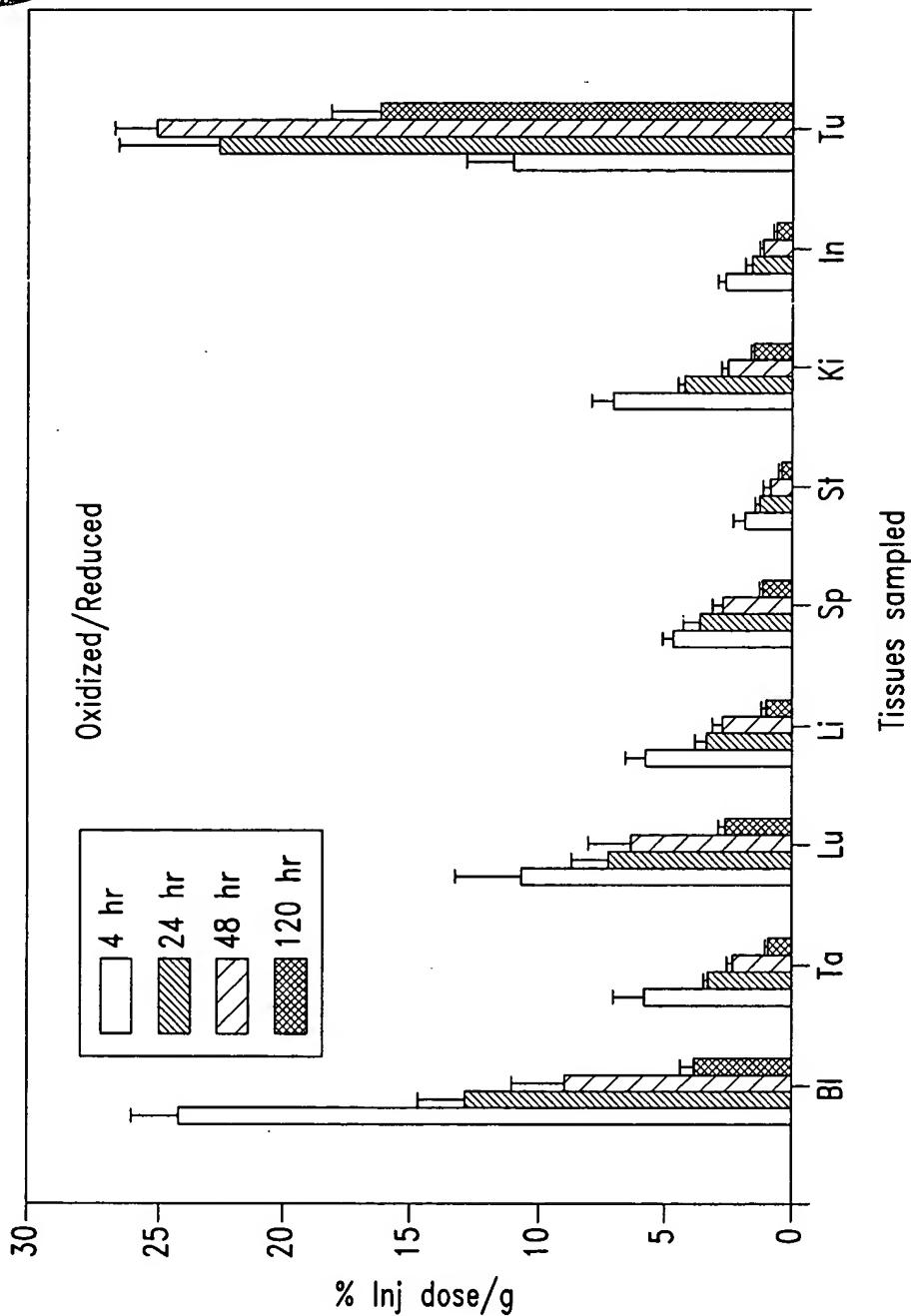


Fig. 16A

10056794 092402

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY

-LU-13 AND THEIR USE IN PRETARGETING METHOD

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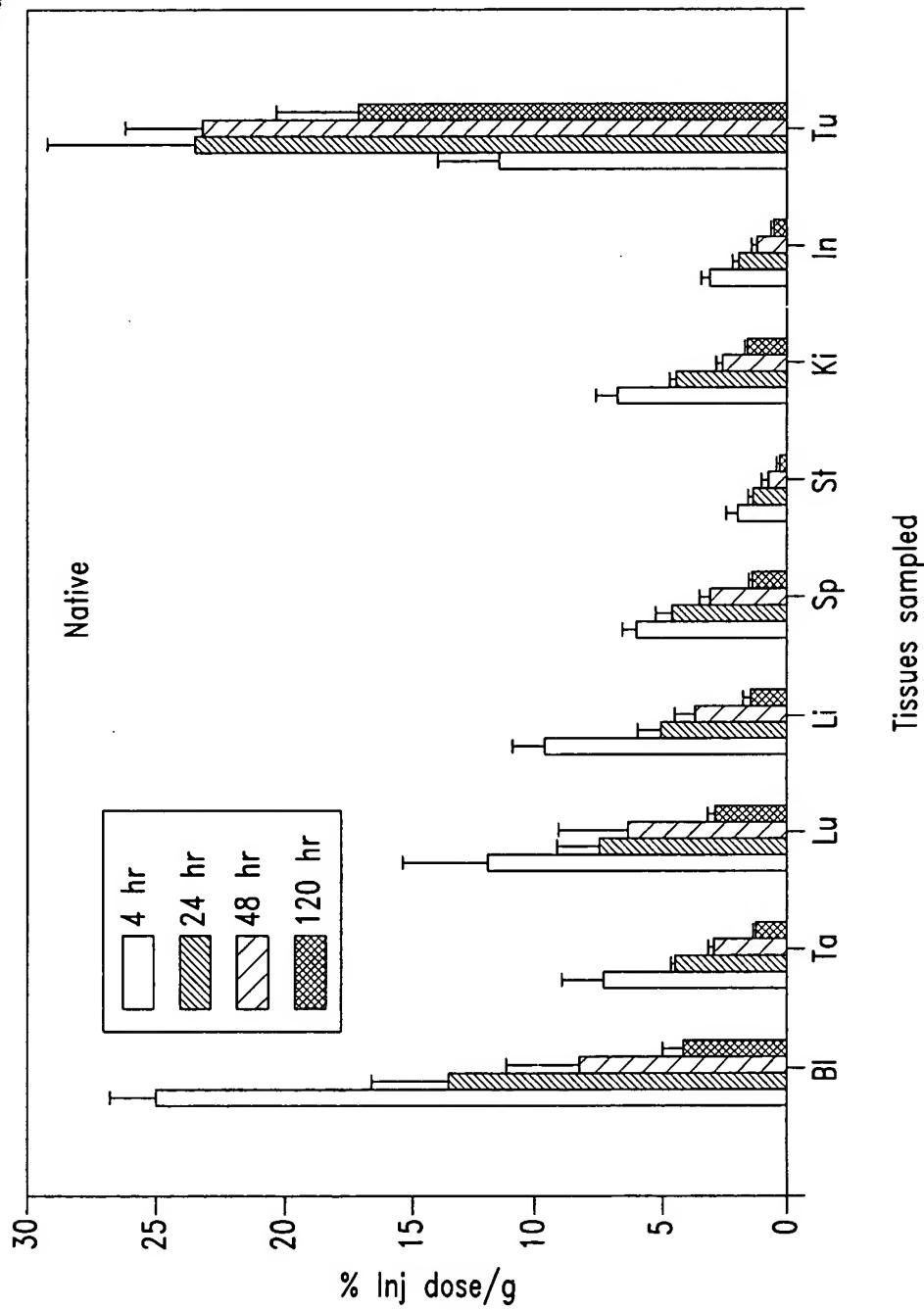


Fig. 16B

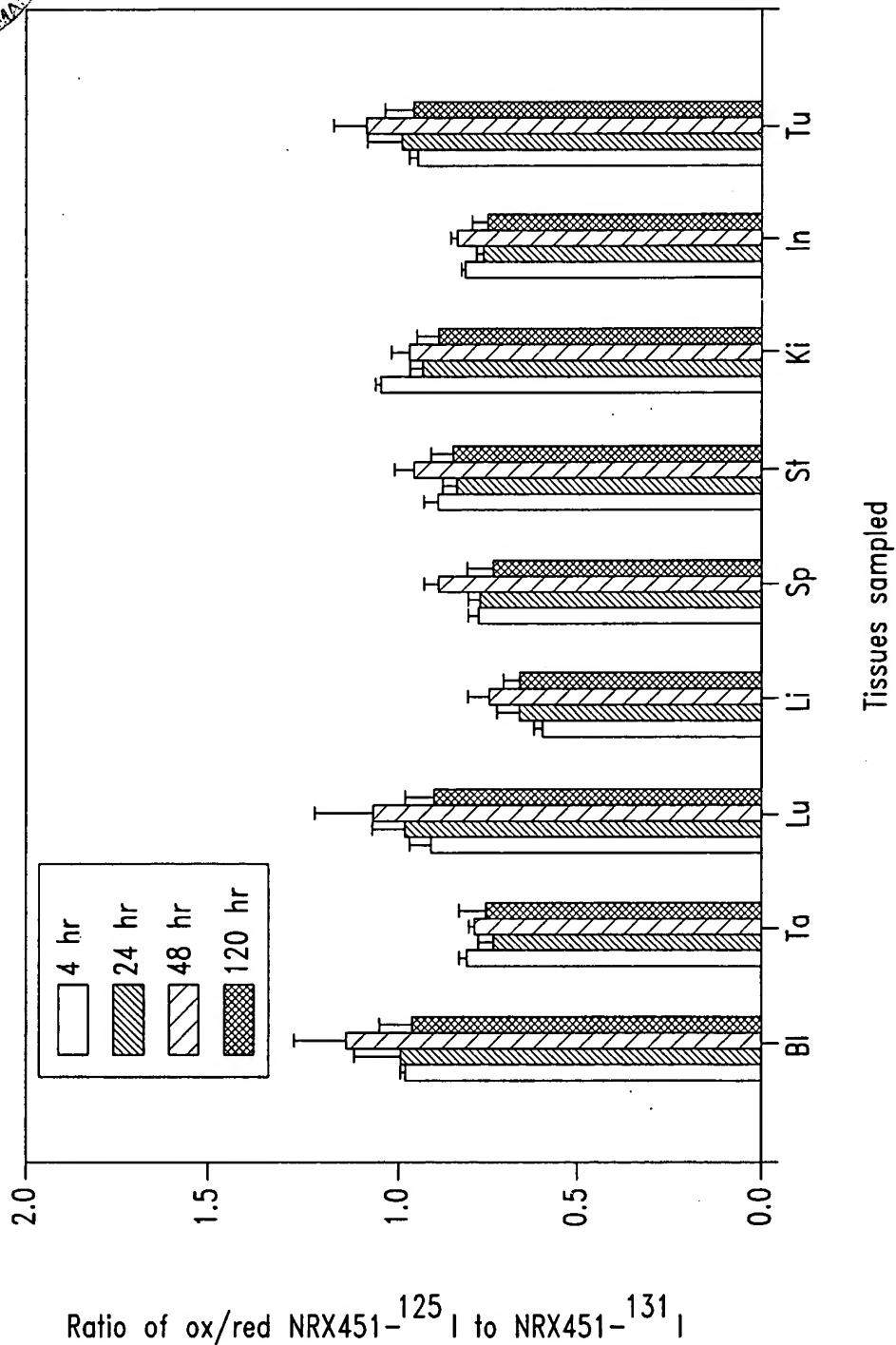


Fig. 16C

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 1056794-052402

-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

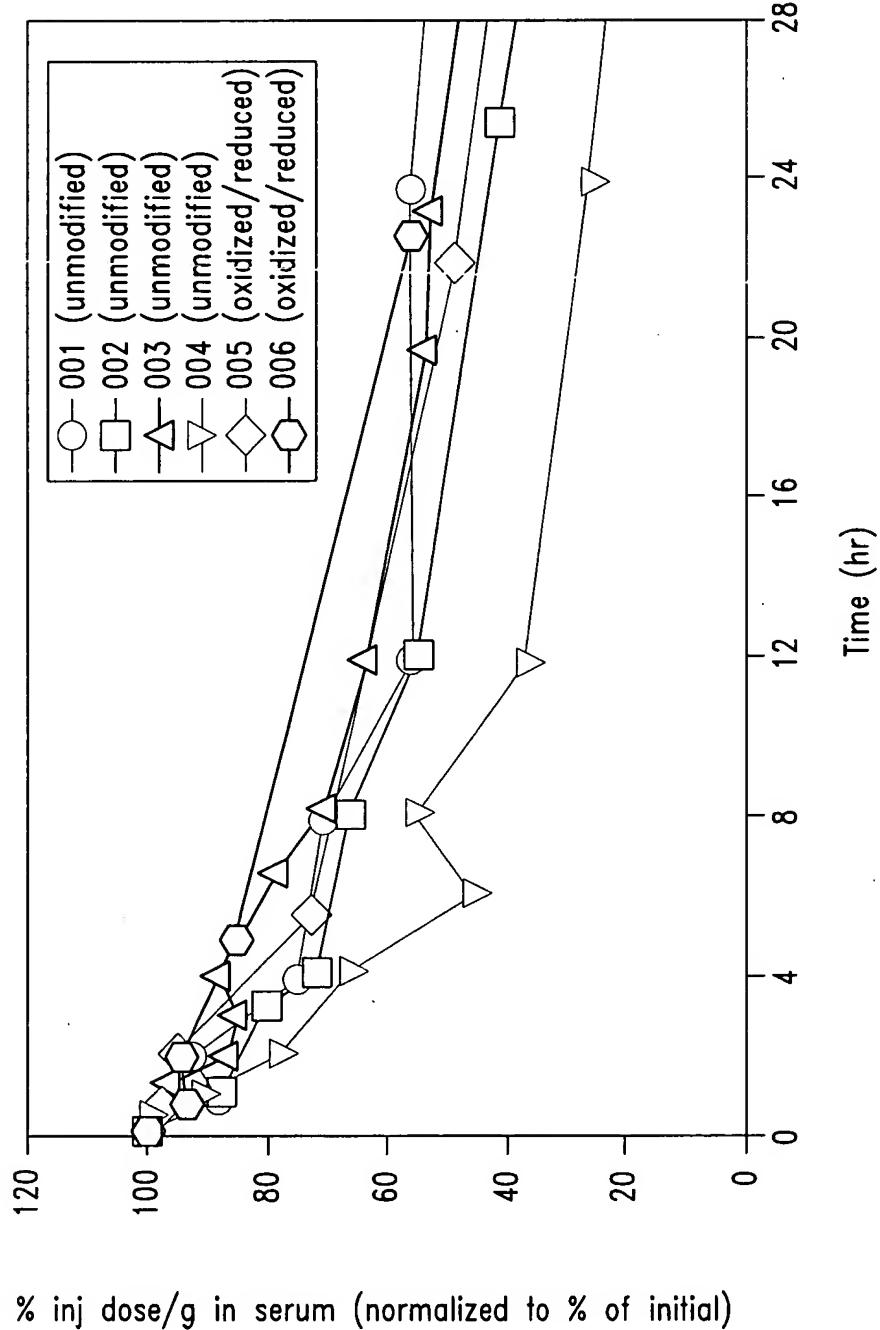


Fig. 17

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 0924402

-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

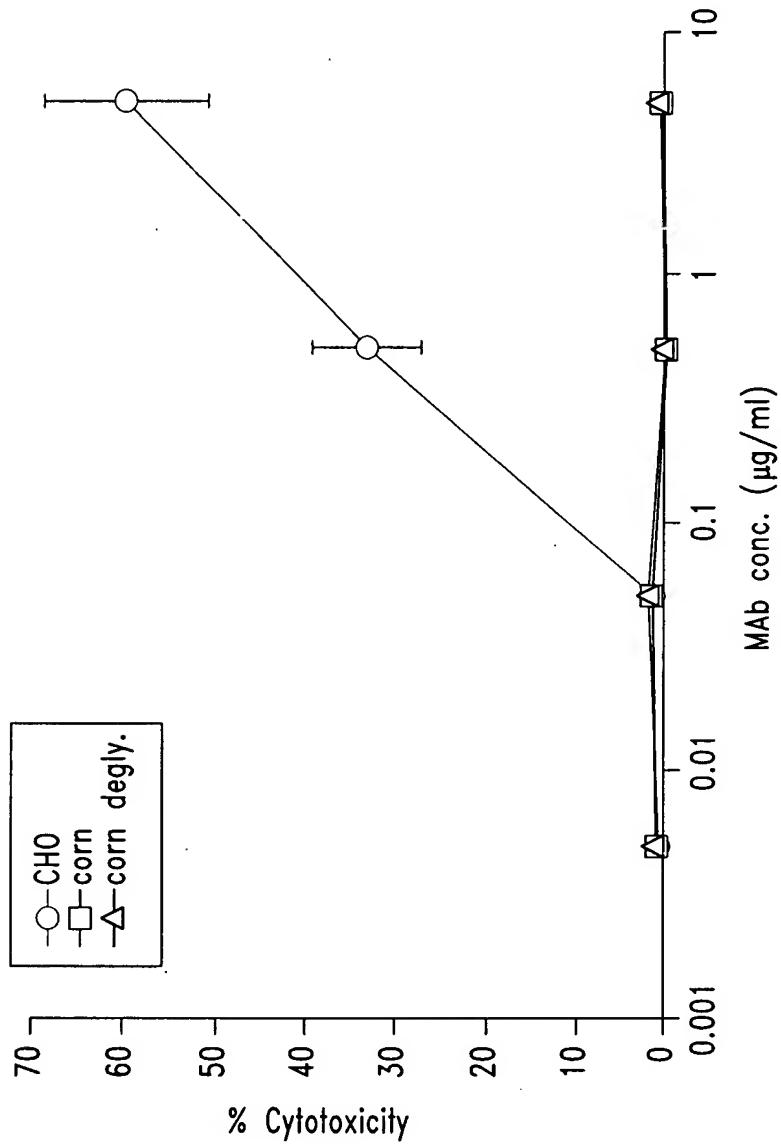


Fig. 18

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY 092402

-LU-13 AND THEIR USE IN PRETARGETING METHODS

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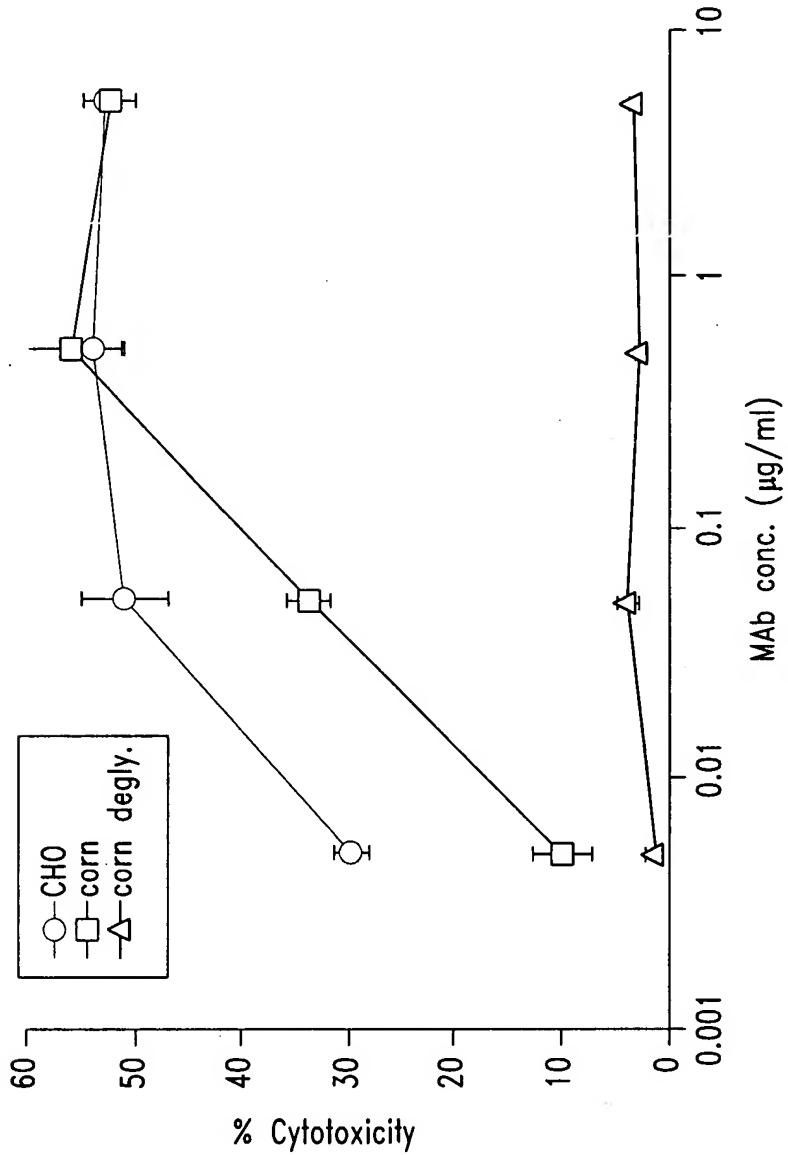


Fig. 19

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY 032402

NR-LU-13 AND THEIR USE IN PRETARGETING METHODS

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

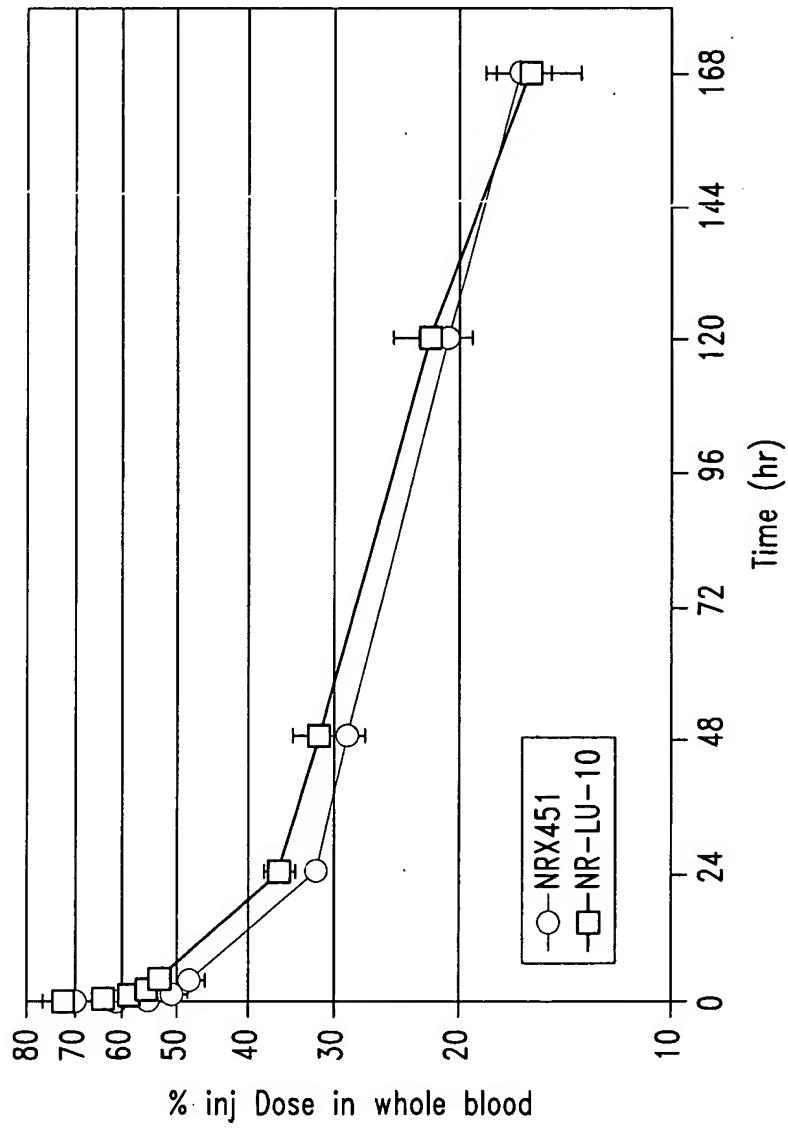


Fig. 20

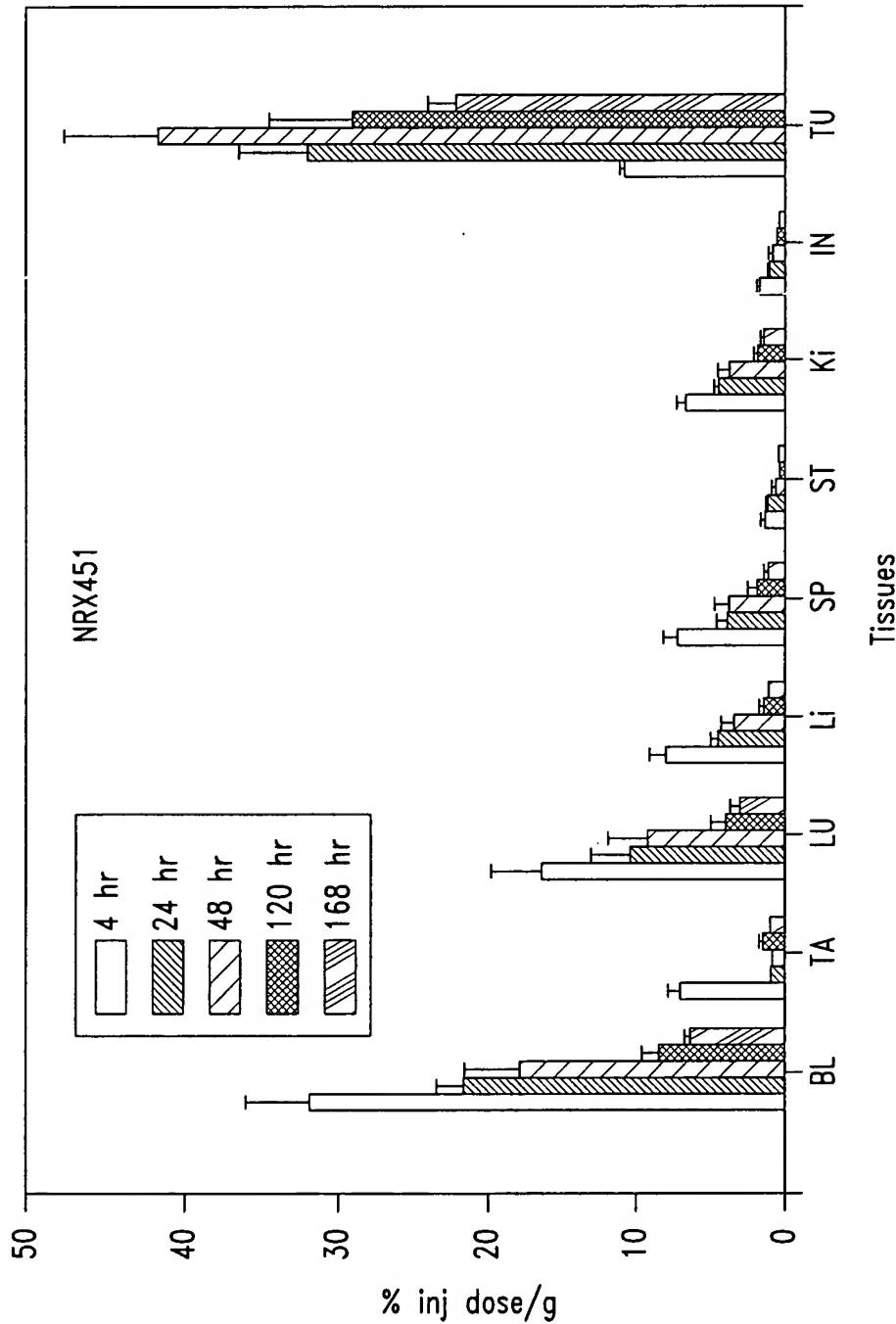


Fig. 21A

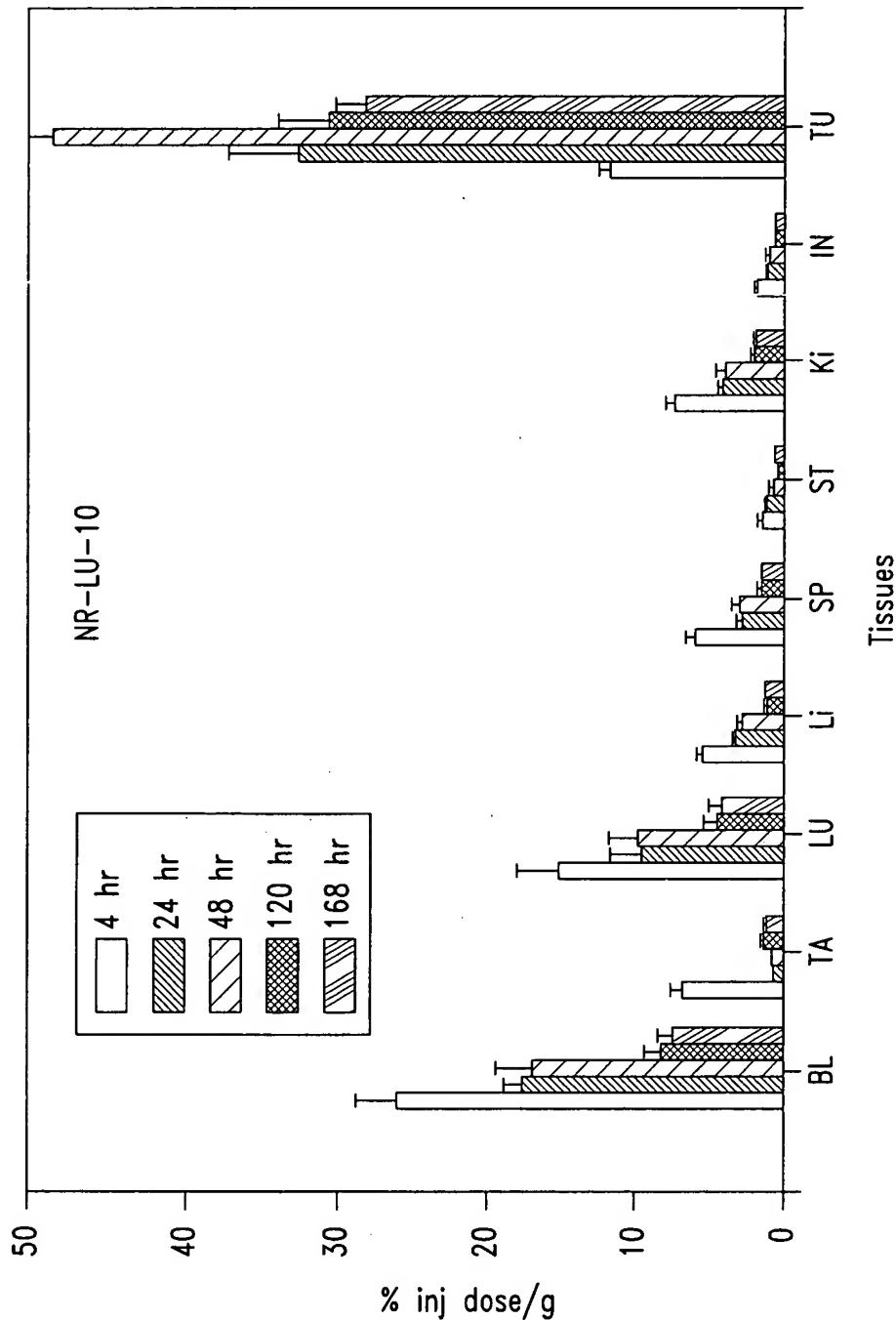


Fig. 21B

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY • 092402

-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

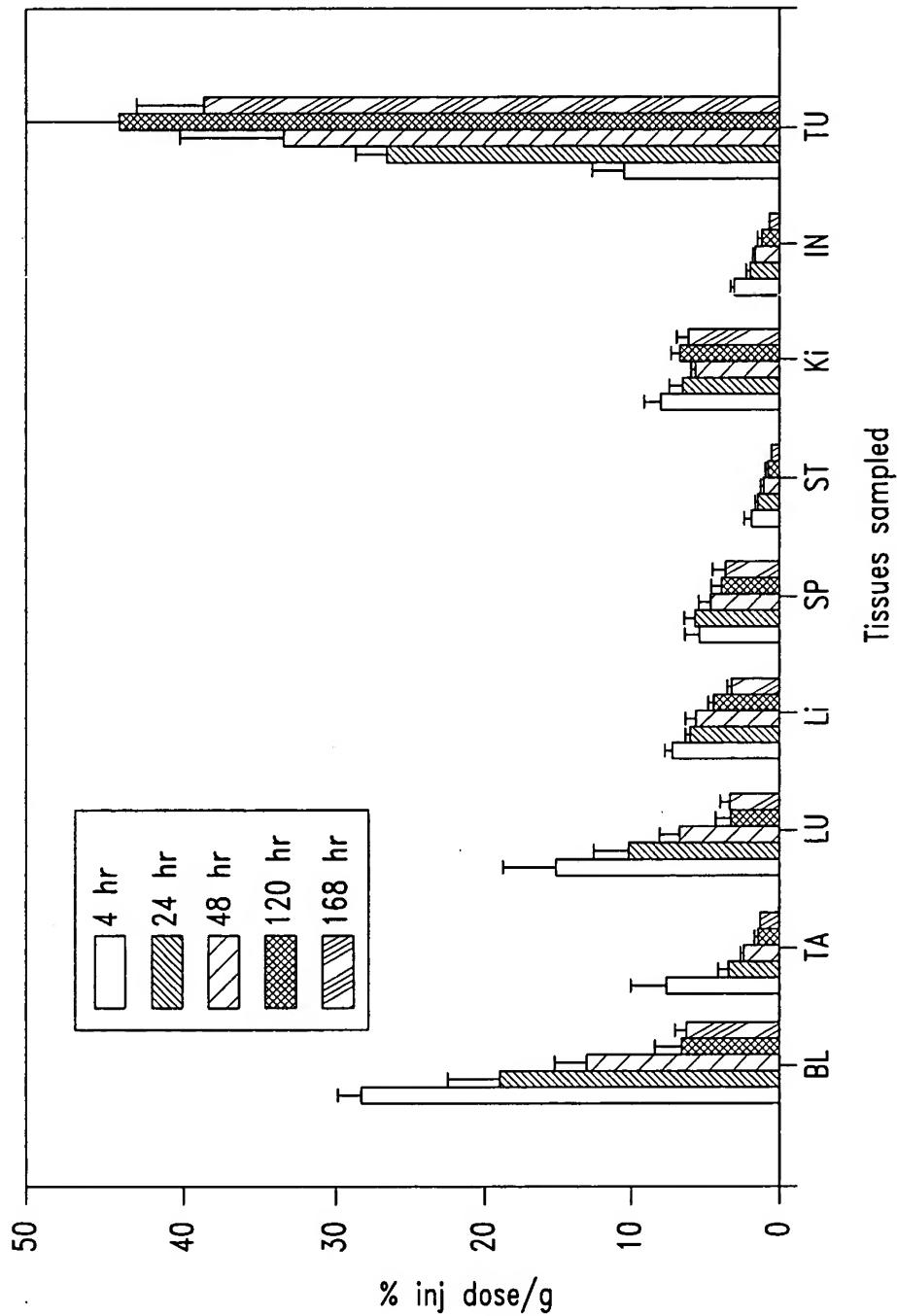


Fig. 22

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUND BY ANTIBODY 10/056,794 - 092402

NRX-LU-13 AND THEIR USE IN PRETARGETING METHODS

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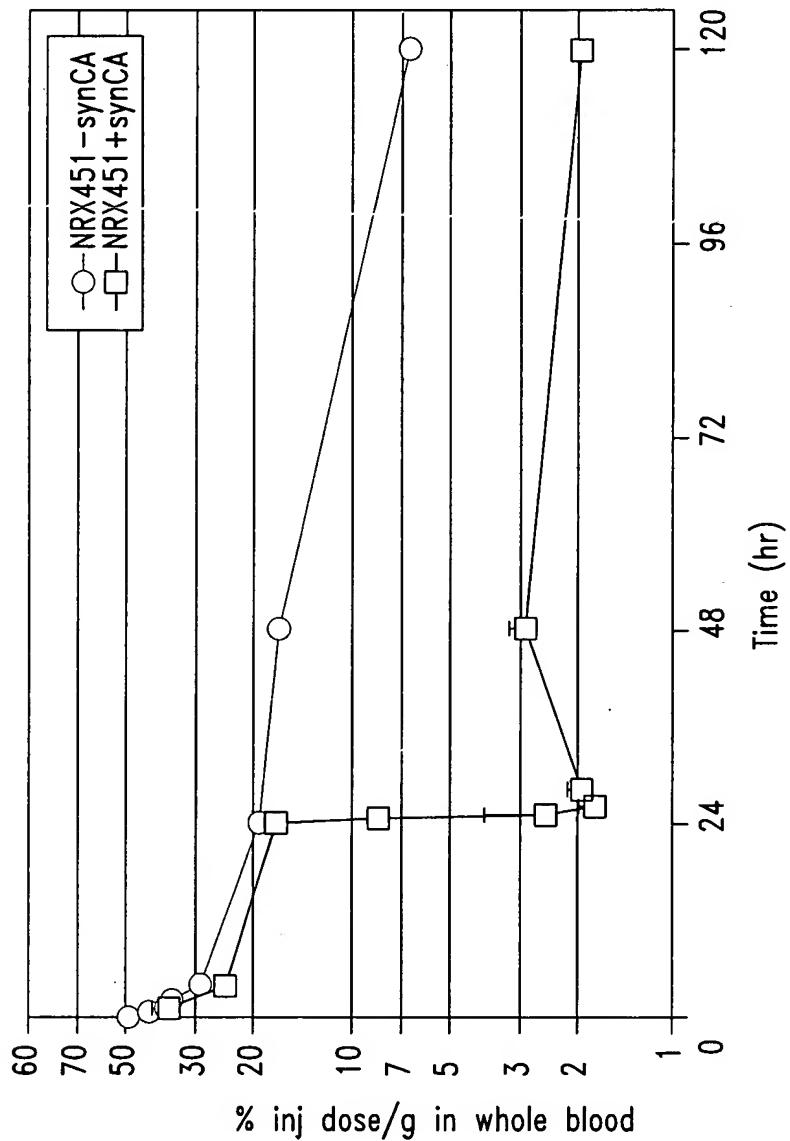


Fig. 23

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BO~~UN~~ND BY ANTIBODY .. 0922402

-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

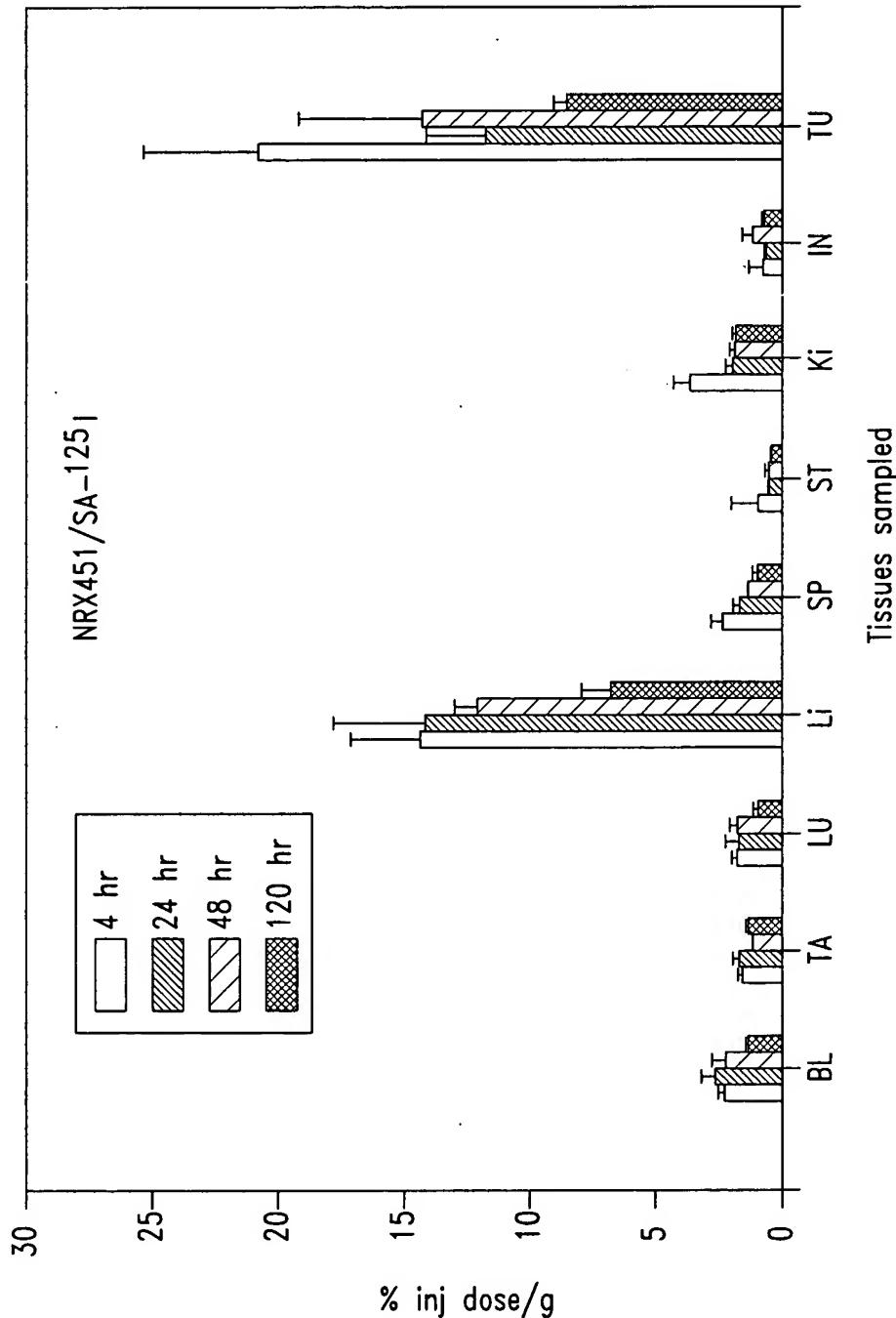


Fig. 24A

Title: HUMANIZED ANTIBODIES THAT BIND TO THE ANTIGEN BOUGHT BY ANTIBODY **1005224402**
[REDACTED]-LU-13 AND THEIR USE IN PRETARGETING METHOD

Inventor(s): Scott S. Graves et al. Serial No. 10/056,794 Docket No. 690022.527C2

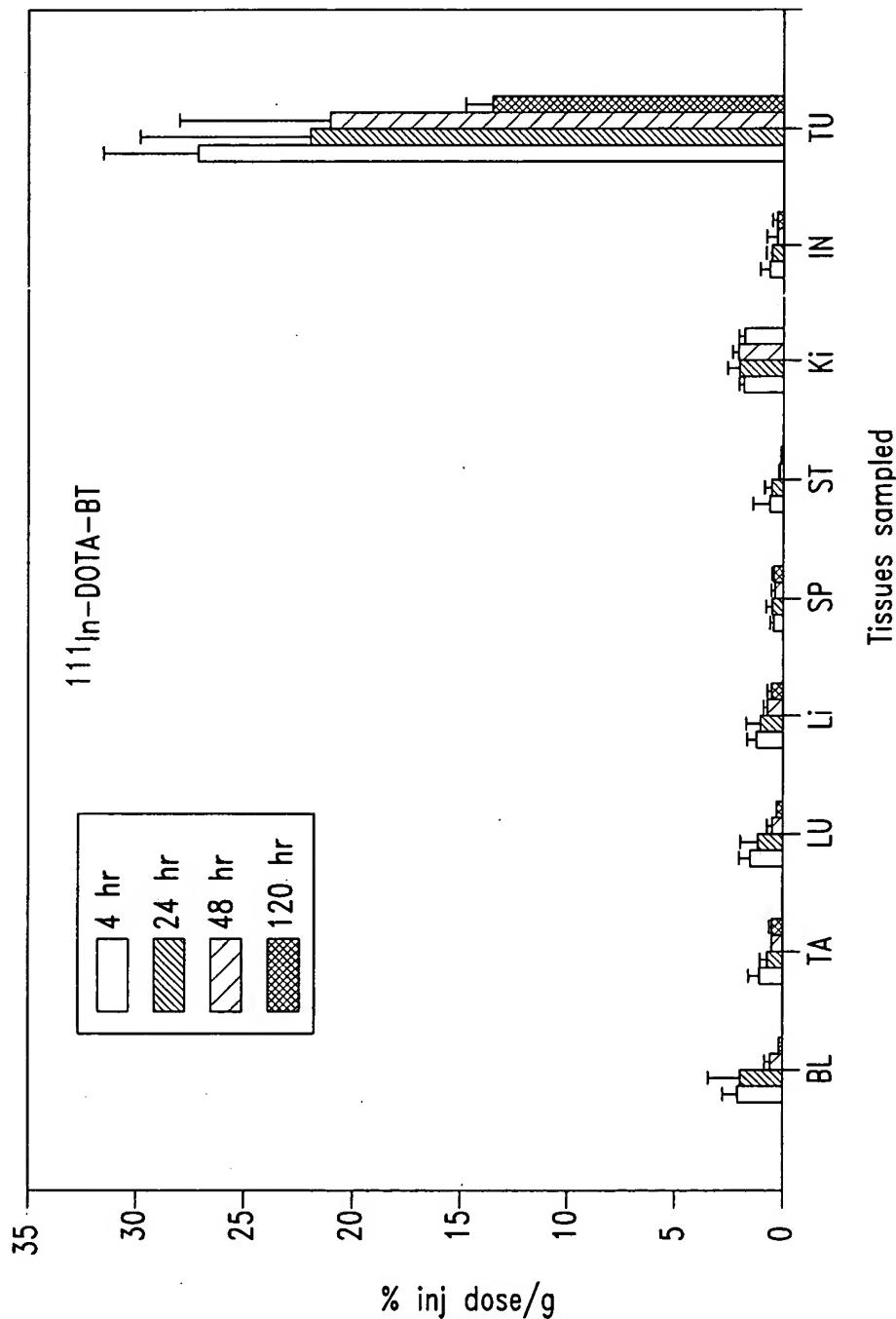


Fig. 24B